DP Barcode: 374484 MRID No.: 479723-06

DATA EVALUATION RECORD
SEED TREATMENT DUST-OFF DISPERSAL- HONEYBEES
(NON-GUIDELINE STUDY)

1. **CHEMICAL:** Clothianidin PC Code No.: 044309

2. **TEST MATERIAL:** Clothianidin Purity: Not reported.

3. **CITATION**

Authors:	Dechet, F., H. Seulberger, M. Lèfevre, P. Neumann, R. Schmuck
Title:	Seed Treatment Dust: Preliminary Results on Dust-off Dispersal, Implement Exposure Mitigation Measures and Experiences with Dust-off under Agronomic Use Conditions. European Industry Presentation to the Bundesamt for Verbraucherschutz and Lebensmittelsicherheit.
Study Completion Date:	November 27, 2009
Laboratory:	Industrieverband Agrar Germany
Sponsor:	Bayer CropScience 2 T.W. Alexander Drive Research Triangle Park, NC 27709 USA
Laboratory Report ID:	G202135
MRID No.:	479723-06
DP Barcode:	D374484

4. **REVIEWED BY:** Joan Gaidos, Senior Scientist, Cambridge Environmental, Inc.

Signature: Joan Gaidos **Date:** 7/14/11

APPROVED BY: John Marton, Staff Scientist, Cambridge Environmental, Inc.

Signature: John Marton **Date:** 8/3/11

5. **APPROVED BY:** {………………………}, {Specialty}, OPP/EFED/ERB-{Section}

Signature: **Date:**

6. STUDY PARAMETERS

Test Species: Honeybees (species not reported).
Age of Test Organism at Test Initiation: Not specified.
Test Duration: Not reported.

7. CONCLUSIONS:

Several studies were conducted under laboratory and semi-field conditions and pilot studies were conducted in France, Switzerland and Austria to investigate the amount and dispersal of fine dust fractions (aerial drift) which does not quickly sediment but may deposit in adjacent vertical vegetation structures like hedgerows or adjacent crop fields; and to determine if exposure to dust particles is more, or less, harmful to bees than foliar sprays

Several studies were conducted comparing sampling devices and drift at and after drilling. The sampling devices hedge, gauze, BSNE (not defined) and pipe cleaner were compared at 0.65 m and 1.65 m distances (not further explained), all reported measurable residue values and reasonable variation. At both measurements (0.65 m and 1.65 m) the lowest residues were collected from the hedge and BSNE samplers at averages of *ca*. 0.2-0.4 mg a.s./m^2, while the gauze and pipe cleaner samplers were averages of *ca*. 0.4 to >0.6 mg a.s./m^2. Dust deposition on scouring pads declined with increasing height above ground with concentrations of *ca*. 4 μg a.s./sample at 1 m above the soil surface and 1 μg a.s./sample at 6 m. Primary drift at drilling plus an additional 30 minutes was the main source of dust dispersal with concentrations of *ca.* 2 and 4 μg/sampler at 1.65 and 0.65 m (not defined). Secondary drift (24 hours or 4 weeks) was measurable but negligible compared to primary drift. Residue levels in the glycerol-water wetted semi-natural proxy hedge were about 5 times higher as in the ground level petri-dishes; supporting data not reported.

In two trails comparing deposition on bare soil with interception by whole plants, the deposition in petri dishes over canopy were always lower than in petri dishes over bare soil. When related to ground area, interception residues by adjacent crop vegetation (from 0.25 m^2) at 1 m distance from the maize field was 2.5-10 times higher than on the petri dishes on bare ground; however, interception residues at 5 and 10 m distances from maize field were below the limit of quantification (not specified). From these deposition studies, the study authors concluded that:

- A robust data set is available for ground deposition in certain crops.
- Pilot studies indicate that exposure to dust drift in closest off-crop vegetation is higher than predicted from classic deposition drift studies, likely due to aggregation of deposition and aerial drift interception (factor 2.5-10).
- The development of a standard protocol for dust drift measurement needs agreement on the following parameters: appropriate and easy-to-handle sampling device, sampling height, sampling duration and drilled area.

- A joint industry database and confirmatory research program to generate more robust exposure data needs to be established and an approach for how to use the data appropriately in risk assessment process needs to be developed.

In the laboratory studies, seed treatment dust (100-200 μm) applied individually to anaesthetized honeybees tended to have a lower honeybee toxicity compared to sprayed products. Seed treatment dust (<500 μm in ground oat flakes) tended to be 2X less toxic in the oral test and 3X less toxic in the residual contact test compared to sprayed product. Supporting data for laboratory tests were not reported.

In the semi-field study (*Phacelia*), total mortality on day 0 was similar for the dust and spray exposure with an additional large mortality spike on day 1 in the x g a.s./ha dust treatment group (not further described). In the semi-field cage studies, results indicated that smaller size fractions of dust seem to reflect the worst case. In the comparison of the small size fraction with the spray application of the corresponding seed treatment formulation resulted in a significantly stronger effect of the fine dust compared to spray, likely due to the more dislodgeable nature of the dust residues. Supporting data for semi-field studies were not reported.

In the Austrian study using corn seed treated with Cruiser and Poncho, 22,500 beekeepers with a total of 320,000 colonies reported bee damage in 31 colonies. In 22 of the 31 reported cases bee damages could be related to maize sowing and residues of seed treatment products were detected on 25 apiaries with a total of 599 colonies. Recorded bee damages temporarily increased losses of adult bees and brood effects, but no colony losses were recorded in these cases.

In the 2008 monitoring study from France, the study authors concluded that sowing with Cruiser-treated corn seed (100,000 ha) caused no suspicious deaths, did not affect colony health and had no impact on overwintering. In the 2009 monitoring study from France, study authors found that sowing with Cruiser-treated corn seed (600,000 ha) caused 2 suspicious mortalities involving a few hundred bees (development of colonies was not impacted). Further investigation found that one incident was due to a high virus infestation and the other attributed to bad agricultural practice leading to an exceptional exposure to dust. Supporting data for these monitoring studies were not reported.

In the Swiss bee monitoring study, dust exposure from the planting operation caused no bee mortalities, residues were not detected in the bees, and researchers concluded that current regulations on the application of corn seeds dressed with clothianidin are adequate under practical conditions. During the guttation phase, there was no increase in bee mortality, residues were not detected in bees or honey and health of the bee colonies were not affected.

The reviewer concludes that the data presented in these various studies and surveys are inadequate to accurately determine the effects of clothianidin-treated maize seedlings on honeybees and colony health. Generally, this document was a summary of pilot studies and surveys with very few details provided as to how the study was conducted and contained no tabular data of results. Seed treatment and test formulation details were not described, test organisms were not described, sampling and analytical methods were not reported, statistical analyses were not described, environmental conditions were not described, the efficacy of the endpoints and the methods for determining the endpoints were not validated, criteria for determining effects were not detailed, and tabular data was not presented to allow independent analysis.

8. ADEQUACY OF THE STUDY

A. Classification: Core/Supplemental/Invalid

B. Rationale:

C. Repairability:

9. GUIDELINE DEVIATIONS: This is a non-guideline test.

10. SUBMISSION PURPOSE: This study was submitted to as a follow-up to 2008 incident in Germany in which it was noted that the potential for dust from seed treatment could harm bees. Several pilot studies were conducted to investigate the amount and dispersal of fine dust fractions (aerial drift) which does not quickly sediment but may deposit in adjacent vertical vegetation structures like hedgerows or adjacent crop fields; and to determine if exposure to dust particles is more, or less, harmful to bees than foliar sprays.

11. MATERIALS AND METHODS

Test Material

The test material was clothianidin, applied as a seed treatment in the formulations known as Cruiser and Poncho (formulation details and storage conditions not reported).

Test Organisms

Colonies of honey bee (species not reported) were used as test units. No further details reported.

Seed Treatment and Crop Maintenance

Seeds treatment and crop maintenance details not provided.

Test Design

Pilot studies were designed to compare several types of sampling devices for the assessment of aerial drift and to compare the amounts of dust collected by various sampling devices with the dust deposition on natural vegetation with artificially increased captivity (i.e. a semi-natural or proxy hedge). The relevant sampling height and sampling duration were also assessed and the practicality and suitability for future use in standardized studies were considered.

Sampling devices compared included: hedge, gauze, BSNE (not defined) and pipe cleaner and 0.65 m and 1.65 m; the distance reported was not further described. Scourer pads were also used as passive samplers but the collected amount could not be related to a m^2 unit. The mean measured dust deposition on scourer pads was measured from 1 to 6 m above soil surface; again, no further details provided. Primary drift, defined as drilling plus additional 30 minutes, was compared to secondary drift, defined as 24 hours to 4 weeks following drilling. Residue levels were also determined on petri dishes at ground level and on a glycerol-water wetted, semi-natural proxy hedge in two trials following sowing of treated maize seeds to compare dust deposition on bare soil with interception by whole plants. Petri dishes were sampled on bare ground, above the OSR (not defined), and whole OSR plant samples from 0.25 m^2.

For all experiments, no further descriptions of sampling devices, number of samples collected or details of methods used were reported.

Additional pilot studies were conducted to determine the effects of exposure to dust deposits compared to spray deposits. Pilot studies were conducted with honeybees to develop experiences with dust application methods under laboratory and semi-field conditions and to determine the effects of dust exposure on non-target arthropods (ongoing study).

In the laboratory, seed treatment dust (100-200 μm) was applied to honeybees, initially on individually anaesthetized honeybees and compared to values obtained from sprayed products (not detailed). Seed treatment dust (<500 μm dispersed in ground oat flakes) was applied to honeybees in an oral and a residual contact test on cherry leaves and compared to sprayed product (not detailed). An extended laboratory study, an exposure of *Chrysoperla* to seed treatment dust (<500 μm dispersed in untreated dust) is ongoing.

In a semi-field study (*Phacelia*), seed treatment dust from a driller (<500 μm, dispersed in ground oat flakes) was applied by hand in a tunnel to *ca*. 20 subplots of *Phacelia* while bees were foraging for nectar and pollen. The design included five treatment groups for control, toxic standard, x g a.s./ha dust, x g a.s./ha spray and 0.2 x g a.s./ha dust (further details and explanations for these treatment groups were not reported).

In a semi-field cage study, caged honeybees were exposed to artificial dust (size fractions <100 µm, 100-200 µm, and 200-400 µm) on plastic petri dishes with bee feeding stations. In another semi-field cage study, bees were exposed to sprayed residues and dust (small size fraction of <100 µm) with *Phacelia* and 3 test concentrations (1X, 4X, and 8X g a.s./ha) and 3 replicates each (5 for the control; further details and explanations for these treatments were not reported).

Approximately 130,000 ha of corn treated with Cruiser and Poncho (not described) were sown in Austria by drilling with an un-modified pneumatic sowing machine with a mandatory seed treatment quality standard of 1.3 g dust per 100,000 seed.

In 2008 and 2009, 100,000 ha and 600,000 ha, respectively, of Cruiser-treated corn seed was sown in France. Deflector use was not compulsory and the minimum seed treatment quality level was 3 g dust/100 kg seed (Ceres test). Monitoring was conducted to compare the health and development of bee colonies placed in regions with low or intensive Cruiser use. The hives were set up before drilling and therefore exposed to dust, guttation fluid and pollen from the treated crop. In 2008, 3 regions containing 32 control and treated sites were monitored; apiaries of 7 hives were located at 12 sites close to fields prior to drilling. Site monitoring included 11 health visits (one pre-flowering) assessing bee population, count of the brood frames, samples for toxicolocal (pollen, bee bread, honey, bees) and pathological analysis (bees and larva); overwinter success was also assessed. In 2009, 6 regions containing 96 control and treated sites were monitored; apiaries of 7 hives were located at 30 sites (210 total hives). Site monitoring included 390 development checks before/after drilling, before/after flowering, and before overwintering to assess residues (bees, pollen, larvae and bee bread; 1050 samples) and pathogens (viruses, parasites, diseases; 480 samples) in adult and bee larvae. Dust emission and desorption was also measured on selected sites (petri dish sampling), active air sampling was also conducted.

In a Swiss bee monitoring study, bee hives were set up in 2 corn fields 6 days prior to sowing with Poncho-treated seeds. The hives were maintained for 7 weeks (trial 1) and 5 weeks (trial 2) after sowing. Residues from bees, pollen honey, and guttation fluid were analyzed, bee mortality was assessed regularly, and colony strength was monitored throughout the study.

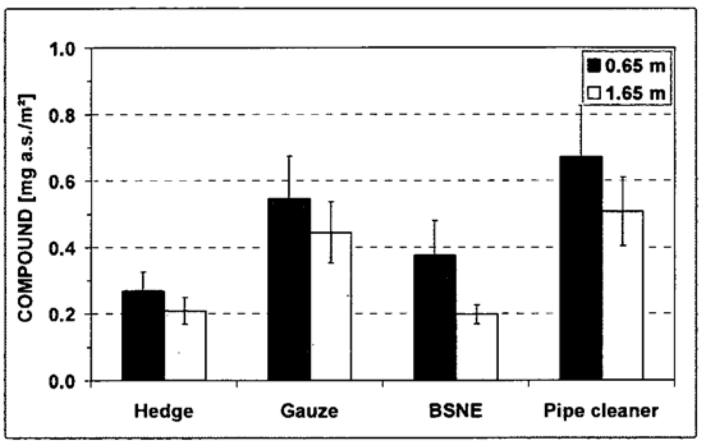
12. REPORTED RESULTS

Signed and dated No Data Confidentiality and GLP statements were provided; a Quality Assurance statement was not provided. This study was not conducted in compliance with the Principles of Good Laboratory Practice (GLP). The study was designed as preliminary results of dust-off dispersal, exposure mitigation measures, and dust-off under agronomic use conditions.

For all results, data points are reviewer estimates from graphs; tabular data was not reported.

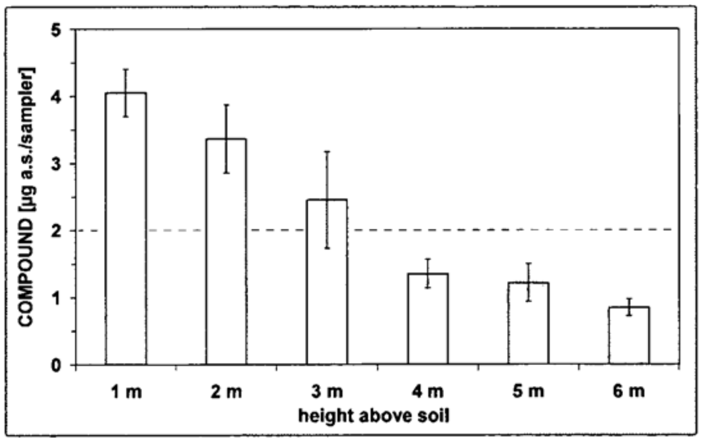
Of the sampling devices compared, all reported measurable residue values and reasonable variation. At both measurements (0.65 m and 1.65 m, not further defined) the lowest residues were collected from the hedge and BSNE samplers at averages of *ca*. 0.2-0.4 mg a.s./m^2, while the gauze and pipe cleaner samplers were averages of *ca*. 0.4 to >0.6 mg a.s./m^2.

<u>Mean residues from sampling devices.</u>



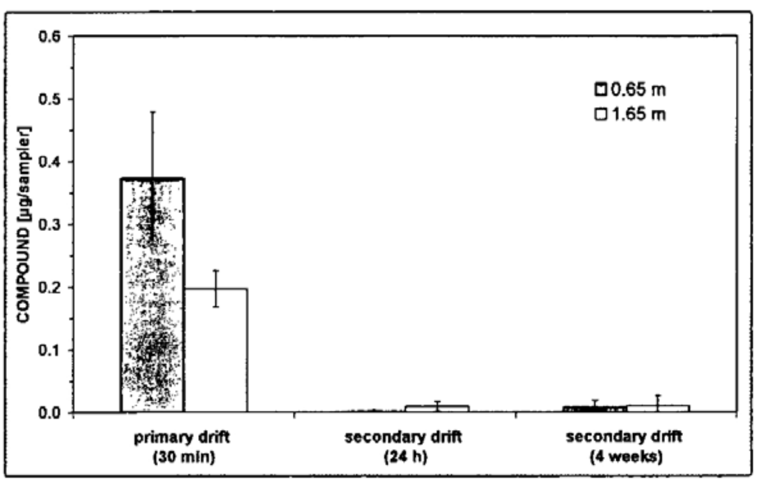
Dust deposition on scouring pads declined with increasing height above ground with concentrations of *ca*. 4 µg a.s./sample at 1 m above the soil surface and 1 µg a.s./sample at 6 m.

Mean measured dust deposition on scourer pads (1-6 m above soil)



Primary drift at drilling plus an additional 30 minutes was the main source of dust dispersal with concentrations of *ca.* 2 and 4 µg/sampler at 1.65 and 0.65 m (not defined). Secondary drift (24 hours or 4 weeks) was measurable but negligible compared to primary drift.

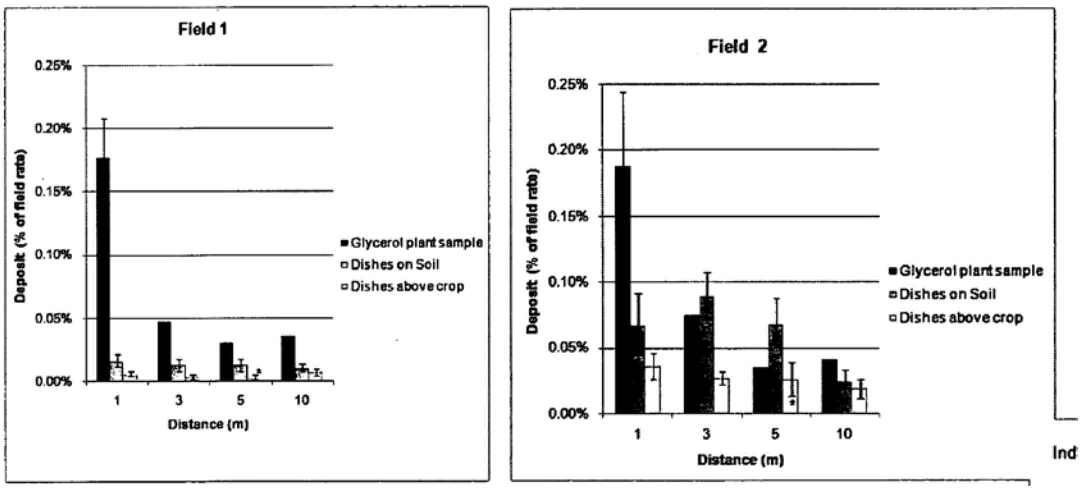
Mean residues associated with drift.



Residue levels in the glycerol-water wetted semi-natural proxy hedge were about 5 times higher as in the ground level petri-dishes; supporting data not reported.

In the two trials comparing deposition on bare soil with interception by whole plants, the deposition in petri dishes over canopy were always lower than in petri dishes over bare soil. When related to ground area, interception residues by adjacent crop vegetation (from 0.25 m^2) at 1 m distance from the maize field was 2.5-10 times higher than on the petri dishes on bare ground; however, interception residues at 5 and 10 m distances from maize field were below the limit of quantification (not defined).

Interception of residues in 2 fields



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- Pilot studies indicate that exposure to dust drift in closest off-crop vegetation is higher than predicted from classic deposition drift studies, likely due to aggregation of deposition and aerial drift interception (factor 2.5-10).
- The development of a standard protocol for dust drift measurement needs agreement on the following parameters: appropriate and easy-to-handle sampling device, sampling height, sampling duration and drilled area.
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Impact of wireworm and corn rootworm damage

For comparison, the study authors reported the financial impact of wireworm and corn rootworm damage at the grower level due to suspension of clothianidin seed treatments in 2009 growing season. Wireworm infestation impacted *ca*. 6% of the total (*ca.* 2 mio hectare) corn acreage resulting in an income decline of 15-30 mio euros. Affected acreage was *ca*. 125,000 ha, other surveys report 75,000 ha. The average decline in yield was *ca.* 18% (other reports average 15-20% declines). Higher impact on grower income from corn crops was prevented due to lifting the suspension of Mesurol for avian deterrence. Also, due to decline in milk products, it is anticipated that growers will convert meadows into cropland, therefore likely creating a higher wireworm pressure in 2010.

Corn rootworm (diabrotica) damage is estimated in the range of 20-90 mio euros accounting for a 10-40% production decline in combination with increased production costs due to soil insecticide applications for an income loss to the grower of 22-90 mio euros. The region of Lombardia reported average yield declines of 5-6% combined with increased production costs due to soil insecticide application (seed treatment 30-38 euros/ha vs. soil insecticide of 60-135 euros/ha) and foliar insecticide application (60-90 euros/ha) resulting in an income decline of *ca.* 20 mio euros at the grower level. Also, the infestation level of corn rootworm has significantly increased and economic damage is expected to be higher next season. Adjacent countries (i.e. Slovenia, Switzerland, France and Austria) will also have an increasing pest pressure due to higher invasion levels (no further details or reference provided).

The study authors summarized:

- In response to the bee incident in 2008, the crop protection industry has established a Joint Industry Stewardship Initiative which succeeded in substantially mitigating the risk posed by dust-off material to honeybees.
- Pilot studies suggest dispersal of dust-off material has a different dispersal pattern in closest adjacent vegetation than shown by ground-based measurements (i.e. petri dishes). These data and their subsequent use in risk assessment require further investigation.
- Dust-off material is more easily dislodgeable than spray deposits translating to potentially higher effects as observed in tunnel tests.
- For evaluating the risk of dust drift to honeybees, bee colony effect field trials and monitoring activities are available to conduct a Tiered risk assessment approach.

Field studies and monitoring activities reported very few effects on honey bee colonies.

- Monitoring studies from drilling treated corn seeds in Austria without the use of deflectors reported a small number of incidents despite a high awareness at beekeeper level. Relative benefits, the scale of impact were considered acceptable by Austrian authorities. The mandatory use of deflectors will further minimize risk to honeybees.
- Monitoring studies in France reported generally healthy bee colonies, no over-wintering effects and only 2 suspicious mortalities (a few hundred bees affected and colony not jeopardized) in >600,000 ha, one due to high virus infestation and the other was attributed to bad agricultural practice.
- Swiss researchers reported no unnatural bee mortalities immediately after sowing and concluded that current regulations on the application of maize seeds dressed with clothianidin are adequate under practical conditions.

Additional study author conclusions:

- Economic damages at the grower level for the 2009 corn season due to suspension of clothianidin seed treatments were substantial.

- Establishment of a joint industry database to further develop technical understanding on dust-off exposure is needed.
- The need for further canopy dust deposition/interception/penetration studies is needed.
- Pilot studies from 2009 need to be completed.
- Further research programs in 2010 are needed to clarify result inconsistencies obtained in the various testing design on effects of dust vs. spray deposit exposure.
- Consideration is needed on how dust deposition data can be incorporated into the risk assessment for honeybees and possibility of modeling approaches considered.

13. REVIEWER'S COMMENTS

The reviewer concludes that the data presented in these various studies and surveys are inadequate to accurately determine the effects of clothianidin-treated maize seedlings on honeybees and colony health. Generally, this document was a summary of preliminary studies and surveys with very few details provided of how the study was conducted and contained no tabular data of results. Seed treatment and test formulation details were not described, test organisms were not described, sampling and analytical methods were not reported, statistical analysis were not described, environmental conditions were not described, the efficacy of the endpoints and the methods for determining the endpoints were not validated, criteria for determining effects were not detailed, and tabular data was not presented to allow independent analysis.

14. REFERENCES: None reported.